REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
2010	Journal Article-Journal of Applied Physiology	

4. TITLE AND SUBTITLE

Effect of hypohydration and altitude exposure on aerobic exercise performance and acute mountain sickness

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Thermal and Mountain Medicine Division
U.S. Research Institute of Environmental Medicine
Natick, MA 01760-5007

8. PERFORMING ORGANIZATION REPORT NUMBER

M10-24

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Same as #7 above.

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Hypoxia often causes body water deficits (hypohydration, HYPO); however, the effects of HYPO on aerobic exercise performance and prevalence of acute mountain sickness (AMS) at high altitude (ALT) have not been reported. We hypothesized that a) HYPO and ALT would each degrade aerobic performance relative to sea level (SL)-euhydrated (EUH) conditions and combining HYPO and ALT would further degrade performance more than one stressor alone and b) HYPO would increase the prevalence and severity of AMS symptoms. Seven lowlander men (25 ± 7 yr; 82 ± 11 kg; mean $\pm$ SD) completed 4 separate experimental trials. Trials were: a) SL- EUH; b) SL-HYPO; c) ALT-EUH; and d) ALT-HYPO. In HYPO, subjects were dehydrated by 4% of body mass. Subjects maintained hydration status overnight and the following morning entered a hypobaric chamber (at SL or 3,048 m, 27°C) where they completed 30-min of submaximal exercise immediately followed by a 30-min performance time-trial (TT). AMS was measured with the Environmental Symptoms Questionnaire-Cerebral Score (AMS-C) and the Lake Louise Scoring System (LLS). The % change in TT performance, relative to SL-EUH, was $-19 \pm 12\%$ (334 ± 64 to 278 ± 87 kJ), $-11 \pm 10\%$ (334 ± 64 to 293 ± 33 kJ), and

15. SUBJECT TERMS

Dehydration; hypobaria; hypoxia; time trial

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			John W. Castellani
Unclassified	Unclassified	Unclassified	Unclassified	9	**19b. TELEPHONE NUMBER** *(Include area code)* 508-233-4953

Reset

Standard Form 298 (Rev. 8/98)
Prescribed by ANSI Std. Z39.18

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Effect of hypohydration and altitude exposure on aerobic exercise performance and acute mountain sickness

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**Castellani JW, Muza SR, Cheuvront SN, Sils IV, Fulco CS,
Kenefick RW, Beidleman BA, Sawka MN.** Effect of hypohydration
and altitude exposure on aerobic exercise performance and acute mountain
sickness. *J Appl Physiol* 109: 1792–1800, 2010. First published September
23, 2010; doi:10.1152/japplphysiol.00517.2010.—Hypoxia often causes
body water deficits (hypohydration, HYPO); however, the effects of HYPO
on aerobic exercise performance and prevalence of acute mountain sickness
(AMS) at high altitude (ALT) have not been reported. We hypothesized that
1) HYPO and ALT would each degrade aerobic performance relative to sea
level (SL)-euhydrated (EUH) conditions, and combining HYPO and ALT
would further degrade performance more than one stressor alone; and *2*)
HYPO would increase the prevalence and severity of AMS symptoms.
Seven lowlander men (25 ± 7 yr old; 82 ± 11 kg; mean ± SD) completed
four separate experimental trials. Trials were *1*) SL-EUH, *2*) SL-HYPO, *3*)
ALT-EUH, and *4*) ALT-HYPO. In HYPO, subjects were dehydrated by 4%
of body mass. Subjects maintained hydration status overnight and the fol-
lowing morning entered a hypobaric chamber (at SL or 3,048 m, 27°C)
where they completed 30 min of submaximal exercise immediately followed
by a 30-min performance time trial (TT). AMS was measured with the
Environmental Symptoms Questionnaire-Cerebral Score (AMS-C) and the
Lake Louise Scoring System (LLS). The percent change in TT performance,
relative to SL-EUH, was −19 ± 12% (334 ± 64 to 278 ± 87 kJ), −11 ±
10% (334 ± 64 to 293 ± 33 kJ), and −34 ± 22% (334 ± 64 to 227 ± 95
kJ), for SL-HYPO, ALT-EUH, and ALT-HYPO, respectively. AMS symp-
tom prevalence was 2/7 subjects at ALT-EUH for AMS-C and LLS and 5/7
and 4/7 at ALT-HYPO for AMS-C and LLS, respectively. The AMS-C
symptom severity score (AMS-C score) tended to increase from ALT-EUH
to ALT-HYPO but was not significant (*P* = 0.07). In conclusion, hypohy-
dration at 3,048 m *1*) degrades aerobic performance in an additive manner
with that induced by ALT; and *2*) did not appear to increase the prevalence/
severity of AMS symptoms.

dehydration; hypobaria; hypoxia; time trial

DURING ACUTE ALTITUDE EXPOSURE (<24 h), a decline in arterial
oxygen pressure, oxygen saturation of hemoglobin, and arterial
oxygen content reduces aerobic exercise performance and
causes acute mountain sickness (AMS) in proportion to the
altitude exposed (26, 32, 34). In addition, high-altitude expo-
sure increases fluid losses (23, 28), which result in body water
deficits (hypohydration, HYPO). Therefore, it is not unusual
for civilian and military personnel to be performing stressful
exercise at high altitude while hypohydrated (7).

The impact of hypohydration on aggravating AMS and
reducing aerobic exercise performance at high altitude has not
been studied experimentally. Epidemiological and field studies
(7, 14) suggest an association between hypohydration and
increased AMS, but this is not well supported by controlled

studies. Hypoxia and hypohydration each reduce aerobic exer-
cise performance independently (26, 23a). For example, max-
imal aerobic power ($\dot{V}O_{2max}$) is reduced by 15% at an altitude
of 3,000 m (18) and by 5% with moderate hypohydration
(∼3% body mass) at sea level (23a). Likewise, aerobic perfor-
mance tests requiring ∼30 min to complete are degraded by
10–15% at an altitude of 3,000 m (18) and by 6–8% during
moderate (3%) hypohydration at sea level (2, 12, 23a). It is
unknown whether combining altitude exposure with hypohy-
dration will exacerbate the prevalence and severity of AMS
symptoms and/or further reduce aerobic exercise performance.

The purpose of this study was to determine the effects of
hypohydration (∼4% body mass loss, hyperosmotic, hypovo-
lemic) on aerobic exercise performance at high altitude and on
the prevalence and severity of AMS symptoms. We hypothe-
sized that hypohydration and altitude exposure would each
degrade exercise performance and that the combination of
these stressors would exacerbate the performance impairment.
We also hypothesized that hypohydration would increase the
prevalence and severity of AMS symptoms. Our approach was
to use a hyperosmotic, hypovolemic model of hypohydration
(−4%), caused by exercise-induced sweating, to mimic the
type and magnitude of hypohydration commonly observed
during recreational activities (climbers, trekkers) and military
operations. To understand the potential effect of hypohydration
on cerebral edema, S100β was measured as a marker of
blood-brain barrier function (3, 4). Furthermore, since de-
creased diuresis has been noted in individuals who are suscep-
tible to AMS (5, 6), fluid-regulatory hormones were measured
to provide insight into possible mechanisms of increased AMS
symptoms. Finally, we used a warm ambient environmental
temperature to replicate the conditions in many high-altitude
regions within 30° of the equator.

METHODS

Subjects. Seven male lowlander volunteers provided written in-
formed consent to participate in this study, which was approved by the
Scientific and Human Use Review Boards of the U.S. Army Research
Institute of Environmental Medicine and the U.S. Army Medical
Research and Materiel Command. The subjects volunteered after
being fully informed of the requirements and risks associated with the
research. Investigators adhered to Army Regulation (AR) 70–25 and
U.S. Army Medical Research and Materiel Command Regulation
70–25 on the use of volunteers in research. Subject characteristics
(mean, SD) were age, 25 ± 7 yr old; height, 176 ± 5 cm; body mass,
82 ± 11 kg; peak oxygen uptake ($\dot{V}O_{2peak}$) at sea level, 44.1 ± 4.9
ml·kg^{-1}·min^{-1}; $\dot{V}O_{2peak}$ at 3,048 m, 38.6 ± 5.5 ml·kg^{-1}·min^{-1}; and
percent body fat, 18.7 ± 3.9%.

Preliminary testing. Several weeks of preliminary tests were com-
pleted before beginning the experimental trials. The first week of
preliminary testing included the measurement of baseline body mass

using a calibrated scale (model WSI-600, Mettler-Toldeo, Columbus, OH). Subject body mass was measured each morning before breakfast and after voiding to establish a reliable baseline for euhydration trials. Body composition and percent body fat were determined from dual-energy x-ray absorptiometry (DEXA, model DPX-L, Lunar, Madison, WI). $\dot{V}O_{2peak}$ was measured, using an incremental test, on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) and a metabolic measurement system (True-Max, ParvoMedics, Sandy, UT). Peak power output (in W) was obtained during this test while the subjects were euhydrated. The $\dot{V}O_{2peak}$ test was conducted twice, once at sea level (SL) and once at an altitude of 3,048 m (ALT).

On a separate day, following completion of the $\dot{V}O_{2peak}$ tests, volunteers cycled, at sea level, for 5–8 min at 100 and 140 W to determine the oxygen uptake ($\dot{V}O_2$) at these power outputs. The relative exercise intensity (relative to $\dot{V}O_{2peak}$ at 3,048 m) was determined for these two power outputs (46% $\dot{V}O_{2peak}$ at 100 W and 62% $\dot{V}O_{2peak}$ at 140 W). The subjects then exercised at higher power outputs to determine the wattage needed at sea level so that the relative exercise intensity at sea level could be matched to the relative exercise intensity at 3,048 m. The power output was also determined that elicited 50% $\dot{V}O_{2peak}$ at SL.

Subjects performed three to four exposure trials (walking at 1.34 m/s in 40°C air temperature using two 50/10 min work/rest cycles) to partially acclimate (~60%) to the heat, enhance cardiovascular stability, and reduce between-subject variability. Subjects also received a 30-min familiarization flight in the altitude chamber to go over safety issues within the chamber and to get an appreciation of ascending and descending from 3,048-m simulated altitude. Before the experimental trials all volunteers practiced the cycle time trial three times to establish a baseline performance time. This ergometer allows for pedal rate-independent (hyperbolic) and -dependent (linear) cycling modes. Linear factors and pedal cadences were determined for each individual as previously described (11). The coefficient of variation for these three familiarization time trials was 3.1 ± 1.8%.

Experimental design. Four separate, counterbalanced experimental trials were conducted with subjects completing all four trials. The volunteer entered a 27.5 ± 0.7°C dry bulb, 6.9 ± 3.4°C dew point environment (27% relative humidity) at either SL or ALT (3,048 m; 10,000 ft) to complete each trial, which was 8 h in duration. SL was defined as an altitude of 152 m (chamber altitude was increased slightly to allow for environmental control). Subjects were blinded to the elevations. Altitude exposure was always followed by a sea-level exposure, and sea-level exposure was always followed by altitude exposure. At least three full days separated each experimental trial (with at least 6 days between altitude trials). Subjects reported the day before each experiment and underwent a hypohydration/euhydration session in the late afternoon hours. These sessions consisted of walking at 1.34 m/s in 50°C air (using 25/5 min work/rest cycles) for 2.5–3 h with and without fluid replacement. Subjects either replaced all the sweat secreted (measured by body mass losses where 1 ml = 1 g) or were given no fluid. The target mass loss in the no-fluid condition was 4%. Subjects slept and then after a light breakfast completed one of the four experimental trials. Two trials (SL and ALT) were performed while euhydrated (EUH) and two trials (SL and ALT) while hypohydrated (HYPO; 4% loss of body mass). Trials are denoted as SL-EUH, SL-HYPO, ALT-EUH, and ALT-HYPO. Figure 1 presents the experimental timeline.

Steady-state exercise and exercise performance test. Two phases (steady state and time trial) were conducted during the exercise portion (60 min total). First subjects completed a 30-min steady-state phase. During this phase, constant-load submaximal exercise was performed in the hyperbolic mode setting that imposes a constant work rate independent of pedaling speed. The 30-min steady-state phase was broken into two workloads: the first workload was 20 min in duration and the second workload was 10 min in duration. Resistance for these two workloads was 100 W (46% $\dot{V}O_{2peak}$) and 140 W (62% $\dot{V}O_{2peak}$) during exercise at 3,048 m. At SL, subjects exercised at the same %$\dot{V}O_{2peak}$, based on the SL $\dot{V}O_{2peak}$ results. Exercise was performed with a fan (3–5 mph) blowing on the subject. The steady-state exercise phase was performed after ~1.25 h of exposure to SL or ALT conditions in the chamber.

After a short break, subjects then began the second phase, a 30-min exercise performance time trial (TT, linear mode). During the TT

4% Dehydration/Euhydration (50°C air, walk/rest for 2.5 - 3 hours)

↓ **Overnight**

Hypobaric Chamber (27°C)

| Sea-Level Euhydrated (SL-EUH) | Sea-Level Hypohydrated (SL-HYPO) | 3048m Euhydrated (ALT-EUH) | 3048m Hypohydrated (ALT-HYPO) |

Exercise From Hour 1-2.5
Submaximal Exercise (30 min)
  20 minutes @ 46% VO_{2peak}
  10 minutes @ 62% VO_{2peak}

Time-Trial (30 min)
  Complete as much work (kJ) as possible

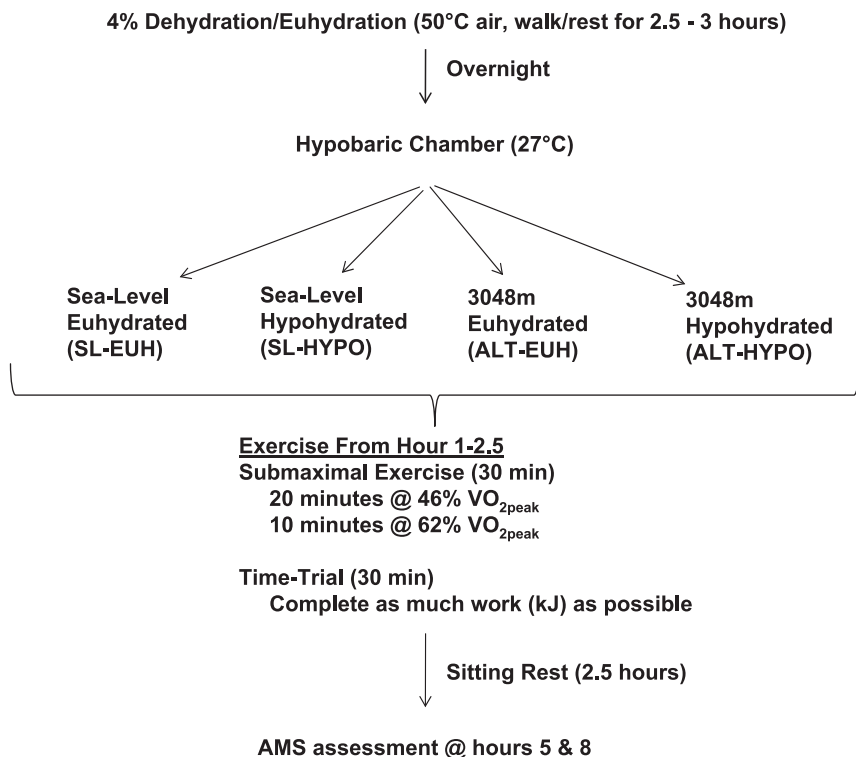↓ **Sitting Rest (2.5 hours)**

AMS assessment @ hours 5 & 8

Fig. 1. Experimental timeline. $\dot{V}O_{2peak}$, peak oxygen uptake.

subjects were blinded to everything except elapsed time, and no motivation was given to the subjects. After completion of the time trial, the subjects were told the amount of work completed (kJ). In the linear mode, work rate increases as pedal rate increases [W = L × (rpm)^2] similar to a mechanically braked cycle ergometer. Individual linear factors (L) were calculated for each volunteer to equal the SL 50% $\dot{V}O_{2peak}$ exercise intensity at a pedal cadence of 60 rpm. The linear factor was kept at the sea level value to maintain the pedal cadence to workload relationship. This avoided the potential problem of dissimilar pedal cadences at equal workloads while preserving the required metabolic cost at any given workload. In the time trial, performance was assessed as the total amount of work completed in 30 min (kJ). Only time was visually displayed for subjects to monitor their progress. Percentage changes in performance, relative to SL-EUH, were calculated to determine the independent and combined stressors of HYPO and ALT. Pacing strategy was also examined. Individual pacing strategy for each trial was calculated by comparing the difference in actual work performed in each of ten 3-min work blocks normalized to the arithmetic mean (total work in 30 min divided by 10). A negative number indicates a slower-than-average pace for that block; a positive number, a faster than average pace.

AMS assessment. AMS assessments were taken in the environmental chamber after 5 and 8 h of exposure during each experimental trial. Subject severity scores that reached the AMS threshold value at either time point were reported as positive for AMS. The prevalence and severity of AMS symptoms was determined from information gathered using a shortened version (8) of the Environmental Symptoms Questionnaire [ESQ (35)] presented on a hand-held personal digital assistant (iPAQ, Hewlett Packard). A weighted average of scores from 11 items (headache, lightheaded, dizzy, etc.) designated AMS-C was calculated. AMS-C scores equal to or greater than 0.7 are defined as diagnostic of AMS. AMS symptoms were also evaluated using the Lake Louise AMS Scoring System (LLS). The LLS consists of a five-question, self-reported assessment of AMS symptoms (33). Total LLS scores ≥ 3 (range, 0–15) that include headache at any time point are diagnostic of AMS.

At the completion of the PDA-based questionnaire, the volunteer's resting arterial oxygen saturation (SaO_2) was measured (Dolphin Medical, Voyager Pulse Oximeter, Hawthorne, CA) for 1 min.

Measurements. Measurements taken during the initial steady-state exercise phase included heart rate (HR, Polar E30, Polar USA, Lake Success, NY), core temperature (esophageal and pill, YSI, Yellow Springs, OH; Philips/Respironics, Bend, OR), skin temperature (YSI; forearm, tricep, chest, thigh, calf; mean skin temperature calculated according to Ref. 29), arterial oxygen saturation, oxygen uptake, local sweat rate, skin blood flow (SBF), blood pressure, ratings of perceived exertion (RPE), and thermal sensation. HR and SaO_2 were also collected during the time trial. During the break period between the steady-state exercise and time trial, body mass was determined and water given in the euhydrated condition so that body mass was equal to the mass measured before the beginning of submaximal exercise. No fluid was given in the hypohydrated condition, except following the TT (to match sweat losses over 60 min of exercise). Esophageal and pill temperatures were not uniformly obtained in every subject on every trial; some subjects who could not insert an esophageal probe on a particular day were given a temperature pill to insert as a suppository.

SaO_2 via noninvasive finger pulse oximetry (model 8600, Nonin Medical, Plymouth, MN) was monitored continuously during the $\dot{V}O_{2peak}$ tests at sea level and 3,048 m and also during exercise in both the steady-state and performance phases. SaO_2 was also determined immediately on finishing the performance portion of the time trial.

RPE [Borg scale (9)] and thermal sensation (19) were measured immediately before and every 10 min during steady-state exercise, and RPE also was measured immediately following the time trial.

SBF (Peri-Med, PeriFlux System 5000) was measured on the ventral forearm (opposite arm of the blood pressure cuff, forearm kept at heart level) using laser-Doppler flowmetry. The flow probe (sampling at 780 nm) was held in place with adhesive, and the temperature underneath the probe was maintained at 33°C. Output (mV) was divided by mean arterial pressure (automated cuff, Cycle model, SunTech Medical, Morrisville, NC) to give an index of cutaneous vascular conductance (CVC, mV/mmHg). Following the time trial, maximal CVC was assessed by locally heating the forearm to 42°C for 30 min. Exercise CVC values were then expressed as a percentage of the maximal CVC, relative to each specific trial.

Local chest sweat rate was measured during the initial 30 min of exercise using a commercially available (Bi-Tronics, model BI102LS, Guilford, CT) ventilated (room air, 1 l/min) dew-point hygrometry system (22). The sensor was held in place with a strap placed around the chest.

Blood and body water. Blood was sampled from an indwelling catheter inserted into a superficial forearm vein and was obtained at two time periods [before altitude exposure (0 h, outside the chamber, 21°C), and at 8 h (8 h) of exposure] after the subject had been sitting for a minimum of 20 min. Blood was analyzed for hemoglobin concentration, hematocrit, and plasma osmolality (freezing-point depression). Hemoglobin and hematocrit were used to calculate %plasma volume (PV) changes (15). Absolute PV was calculated from fat-free mass from the equation of Sawka et al. (38) for both EUH trials. To calculate the absolute PV for the HYPO trials, the %ΔPV at 0 h, relative to EUH, was determined using the Dill and Costill equation (15) and absolute PV adjusted for the HYPO trials for this change. Initial total body water (TBW) was calculated as 0.74 × lean body mass + 0.10 × fat mass as this relationship is constant in adults (23a). The change in TBW was calculated by the change in body mass for each trial from the initial pre-HYPO body mass.

Plasma renin activity (PRA), aldosterone (ALD), arginine vasopressin (AVP), and atrial natriuretic peptide (ANP) were measured before exposure (0 h) and after 8 h of exposure. PRA was measured with a commercially available two-site immunoradiometric assay (DSL-25100 ACTIVE Renin IRMA kit, Diagnostic Systems Laboratories, Webster, TX). The %CV for PRA was 4.5% (intra-assay) and 6.8% (interassay). ALD was measured with a commercially available radioimmunoassay (RIA, DSL-8600 ACTIVE kit, Diagnostic Laboratories, Webster, TX). The %CV for ALD was 5.9% (intra-assay) and 3.7% (interassay). AVP was measured with a commercially available double-antibody RIA (Buhlmann Laboratories AG, Schonenbuch, Switzerland). The intra-assay %CV for AVP was 17.9%. ANP was measured using a commercially available non-equilibrium RIA (Euro-Diagnostica, Malmo, Sweden). The %CV for ANP was 18.2% (intra-assay) and 12.9% (interassay). S100β was measured before and after 8 h of exposure as a marker of blood-brain barrier integrity (24). S100β was measured using a commercially available enzyme-linked immunosorbent assay (ALPCO Diagnostics, Salem, NH). The %CV for S100β was 4.3% (intra-assay) and 6.8% (interassay). S100β was used as a marker of possible blood-brain barrier disruption and has been previously measured in hypoxia studies to potentially explain cerebral edema (3, 4).

Statistical analysis. Total work, HR, SaO_2, body mass, RPE, SBF, and blood measurements were analyzed using a three-way (altitude × hydration × time) repeated-measures analysis of variance (RMANOVA). AMS severity scores were analyzed using two-way (hydration × altitude) RMANOVA. The Newman-Keuls procedure was used when significant differences among means were identified using RMANOVA. Data are presented as means ± SD. The relative risk of developing AMS during altitude exposure was determined from the prevalence rates in the EUH and HYPO trials (prevalence rate in HYPO divided by prevalence rate in EUH).

For the time-trial performance, the practical importance of these differences was examined by comparing the mean and 95% confidence intervals against an a priori zone of indifference, defined as the %CV observed during performance training (calculated from famil-

iarization *trials 2 and 3* and equal to 3.1%). This procedure affords evaluation against a standard other than zero, is similar to equivalence testing in the clinical sciences (16), and affords determination of whether a performance change is of practical or meaningful importance. As well, the %change from SL-EUH for the other three trials was analyzed using a one-way RMANOVA.

The onset of chest sweating rate was determined by segmented linear regression, a method of regression analysis that identified the intersection of two line segments formed when a breakpoint occurred in the chest sweating rate as a function of time (10).

The primary outcome variable in this study was the amount of work completed during the performance time trial. A power analysis at $\alpha <$ 0.05 and $\beta = 0.20$ indicated that seven to eight subjects would provide sufficient power to detect a meaningful difference (6% change in time-trial performance; $\sim$20 kJ) among trials assuming a mean total work of $\sim$330 kJ and a CV of 3.1%. The desire to detect a twofold change from the %CV was chosen based on the likelihood of experimental perturbations (HYPO, ALT) producing practical or meaningful performance changes from SL-EUH (11).

RESULTS

Hydration level. Markers of hydration status are presented in Table 1. Volunteers were $-4.0 \pm 0.4\%$ hypohydrated in the HYPO trials and $-0.6 \pm 0.5\%$ hypohydrated in the EUH trials. Body masses were maintained at these levels throughout the 8-h exposure. Measurements of TBW, hematocrit, hemoglobin, PV, and plasma osmolality were consistent with hypohydration. HYPO resulted in a reduction in TBW (6–7%) and PV (10–12%), and an increase in plasma osmolality (Table 1). No differences were observed between SL and ALT during the HYPO trials.

Time-trial performance. Table 2 presents individual and mean time-trial performance data. Total work was lower in SL-HYPO compared with SL-EUH, and ALT-HYPO was significantly lower vs. the other three trials. One subject was unable to complete the entire 30-min bout during both HYPO

Table 2. *Time-trial work performance*

Subject No.	SL		ALT	
	EUH	HYPO	EUH	HYPO
1	281.4	179.6	280.9	57.6
2	364.2	331.5	320.7	288.7
3	254.4	165.2	225.6	155.8
4	353.1	302.1	292.8	215.7
5	292.8	255.7	305.4	242.1
6	348.9	291.4	301.9	289.9
7	443.9	417.0	323.9	338.2
Mean	334.1	277.5*	293.0	226.8†
SD	63.7	87.4	33.3	95.1

Values are time-trial work performance in kJ. *Significantly lower ($P <$ 0.05) than SL-EUH. †Significantly lower than SL-EUH, SL-HYPO, and ALT-EUH.

trials. ALT-EUH performance was lower than SL-EUH but did not reach statistical significance using a RMANOVA ($P = 0.06$). Individual performance changes, relative to SL-EUH, demonstrated that 6/7 volunteers did worse during ALT-EUH, while 7/7 subjects did worse during SL-HYPO and ALT-HYPO. Figure 2 presents the percent change in performance, relative to SL-EUH, for ALT-EUH, SL-HYPO, and ALT-HYPO. Time-trial performance was $-11.0 \pm 10.4\%$ worse for ALT-EUH, $-18.6 \pm 12.2\%$ worse for SL-HYPO, and $-33.7 \pm 22.3\%$ worse for ALT-HYPO. The confidence interval for SL-HYPO and ALT-HYPO falls entirely outside the a priori zone of indifference, which provides evidence that the negative effect of HYPO and ALT on performance in warm air is also of practical importance.

To evaluate pacing strategies, the average power output (PO, in W) over the entire 30-min time-trial period ($n = 6$) was calculated. Average PO was SL-EUH, 193.3 ± 57.0; SL-HYPO, 168.3 ± 64.5; ALT-EUH, 166.7 ± 44.5; and ALT-

Table 1. *Hydration markers before and after hypohydration*

	SL		ALT	
	EUH	HYPO	EUH	HYPO
Body mass, kg				
Pre-HYPO	82.6 (10.1)	83.4 (10.1)	82.6 (10.1)	83.0 (10.0)
0 h	82.1 (10.0)	80.1 (9.6)	82.1 (10.4)	79.6 (9.7)
8 h	81.7 (9.9)	79.7 (9.5)	81.7 (10.3)	79.4 (9.6)
Total body water, liters				
Pre-HYPO	51.1 (5.4)	51.6 (5.3)	51.1 (5.4)	51.3 (5.3)
0 h	50.6 (5.3)	48.3 (4.9)	50.7 (5.7)	47.9 (5.0)
8 h	50.2 (5.1)	47.9 (4.7)	50.2 (5.6)	47.8 (4.9)
Hematocrit, %				
0 h	41.7 (2.7)	44.5 (2.4)	42.3 (2.5)	45.5 (3.1)
8 h	42.9 (2.0)	45.8 (2.0)	43.5 (3.0)	46.4 (3.0)
Hemoglobin, mg/dl				
0 h	14.2 (0.9)	15.1 (0.8)	14.4 (0.9)	15.5 (1.0)
8 h	14.6 (0.9)	15.6 (0.7)	14.7 (1.0)	15.8 (1.0)
PV, liters				
0 h	3.37 (0.31)	3.05 (0.33)	3.37 (0.33)	2.96 (0.36)
8 h	3.27 (0.45)	2.90 (0.32)	3.21 (0.34)	2.86 (0.37)
%ΔPV	-3.4 (5.1)	-4.9 (4.3)	-4.9 (2.7)	-3.4 (7.0)
DPV, ml	104 (180)	152 (145)	164 (86)	104 (214)
Plasma osmolality, mOsm/kgH$_2$O				
0 h	290.9 (5.1)	298.1 (5.0)	291.2 (6.0)	301.2 (6.7)
8 h	289.2 (3.6)	297.4 (7.6)	289.1 (3.9)	296.0 (4.3)

Values are means (SD). SL, sea level; ALT, 3,048-m altitude; EUH, euhydration; HYPO, hypohydration. Pre-HYPO is measurement before hypohydration procedure began; 0 h is measurement before exposure; 8 h is measurement after 8 h of exposure. PV, plasma volume.
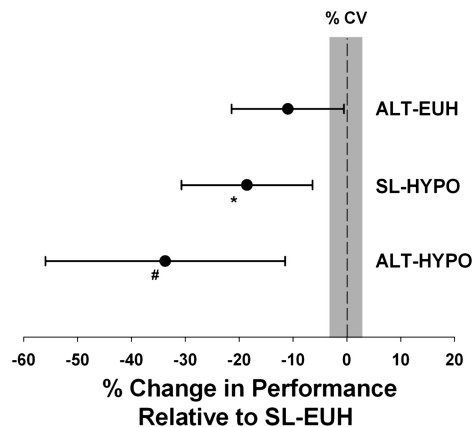
Fig. 2. Percent change in performance from euhydration (EUH) in hypohydrated (HYPO) and altitude (ALT) trials. Data are means; bars are 95% confidence intervals. Shaded area represents the coefficient of variation (CV, ±3.1%) based on the performance variability during the practice sessions. SL, sea level. *Denotes difference ($P = 0.04$) from SL-EUH; #Difference ($P < 0.001$) from SL-EUH. Bars represent the mean ± 95% confidence interval.

HYPO, 145.0 ± 53.2. Analysis of the pacing strategy (Fig. 3) indicated that during the last 3 min of the time trial (end spurt where subjects are trying to get in as much work as possible), subjects' pace was above the normalized average, but that this pace was lower at ALT vs. SL (main effect, $P < 0.02$). There was also a tendency throughout the time trial to have a lower normalized pace average when HYPO compared with EUH, but this was not statistically significant ($P = 0.07$).

AMS. Individual AMS-C and LLS scores are shown in Fig. 4. In general, both the severity of AMS-C and LLS scores and the number of subjects reporting AMS tended to increase in ALT-HYPO. However, there was no statistically significant difference between ALT-EUH and ALT-HYPO for severity scores using the AMS-C score ($P = 0.07$). For both the AMS-C and LLS, the symptom severity score increased ~2.5 times from ALT-EUH to ALT-HYPO. Using the AMS-C score, in ALT-EUH, two subjects (29%) reached the AMS symptom threshold, whereas in the ALT-HYPO trial, five subjects (71%) met the threshold value of 0.7 for AMS symp-
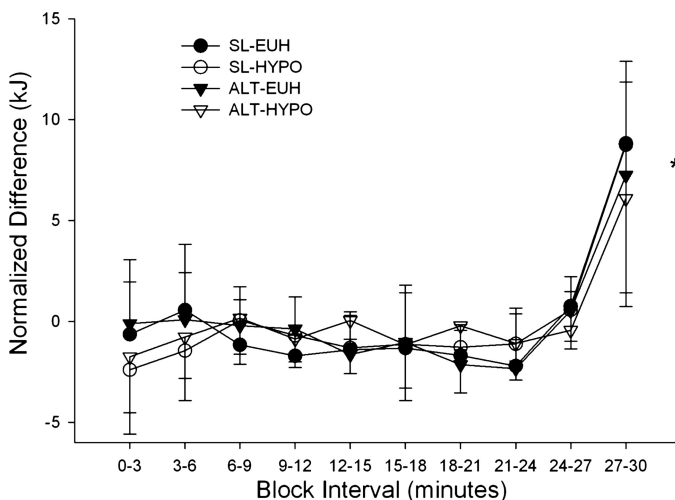

Fig. 3. Differences in pacing strategies between actual and normalized blocks of work recorded at 3-min intervals over 30-min time trial. *Main effect of ALT less than SL ($P < 0.02$).
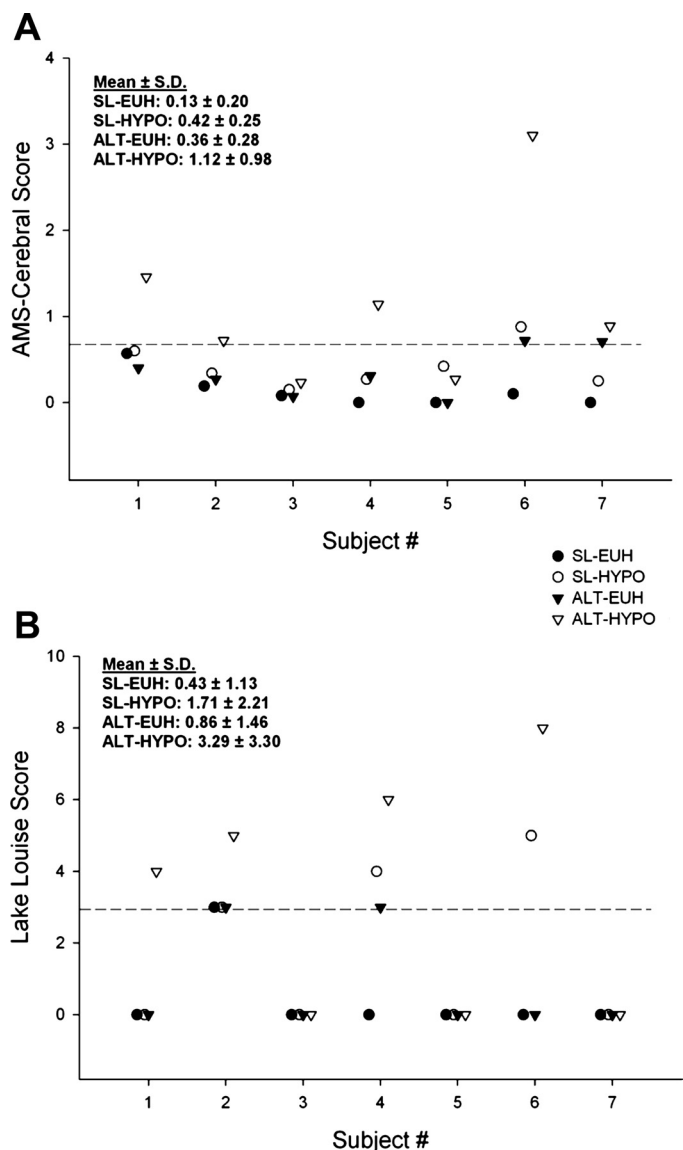

Fig. 4. Individual acute mountain sickness (AMS)-cerebral (*A*) scores and Lake Louise Scores (*B*) after exposure to SL, HYPO, and ALT. Each trial is denoted by one symbol. The threshold for AMS for each scale is denoted by the dotted line. Values for each subject were offset for viewing individual symbols.

toms (2.5 times increase in relative risk). For the AMS-C score, headache was identified in 2/7, 3/7, 2/7, and 2/7 volunteers for SL-EUH, SL-HYPO, ALT-EUH, and ALT-HYPO, whereas for the LLS, headache identification and AMS prevalence was 1/7, 3/7, 2/7, and 4/7. SaO$_2$ levels after 8 h of exposure were 96.9 ± 0.5%, 96.5 ± 0.7%, 89.9 ± 2.2%, and 89.9 ± 2.1% for SL-EUH, SL-HYPO, ALT-EUH, and ALT-HYPO, respectively. ALT trials had a lower SaO$_2$ than SL ($P < 0.001$), with no difference between hydration levels.

Physiological and perceptual responses to submaximal and time-trial bouts. HR values during exercise were higher during HYPO vs. EUH (main effect, $P < 0001$), both during submaximal and time-trial exercise, but there were no differences between SL and ALT (Fig. 5). HR values at the end of the time trial, when volunteers were working to complete as much work as possible, were 171.3 ± 20, 175.3 ± 24.4, 165.8 ± 20.7, and

172.3 ± 19.7 beats/min for SL-EUH, SL-HYPO, ALT-EUH, and ALT-HYPO, respectively ($P > 0.05$). SaO$_2$ levels during exercise were lower ($P < 0.0001$) in both ALT trials (81.0–82.5%) compared with SL (94.6–95.8%), but there were no differences between EUH and HYPO. (Fig. 5)

RPE was not different between SL and ALT during submaximal exercise but was significantly higher during HYPO vs. EUH ($P < 0.05$). At the end of the time trial, RPE was the same among trials (ranged from 18.7 ± 1.4 to 19.0 ± 1.3). Thermal sensation increased with time, but there were no differences between hydration levels or altitude conditions.

Thermal variables are presented in Table 3. Core temperature at the end of the submaximal exercise was ~0.4°C higher in both HYPO trials, compared with EUH, but there was no difference between altitudes. Statistics were not run on core temperature due to the different measurement modes obtained on the subjects. Mean skin temperature decreased from *minutes 0 to 10* and plateaued throughout the rest of the trials. There was a significant time-by-hydration interaction ($P < 0.02$) where HYPO trials had a 0.25–0.40°C higher mean skin temperature compared with EUH trials throughout submaximal exercise. The onset of chest sweating (in min, $n = 5$) was later

($P < 0.05$) in ALT-HYPO (9.5 ± 3.9 min) compared with SL-EUH (3.7 ± 2.6 min), SL-HYPO (5.9 ± 1.9 min), and ALT-EUH (5.0 ± 1.2 min). There were no differences among the latter three trials. CVC, expressed across time, was not different among the four trials.

Total fluid intakes (ml) over the 8-h exposure were 893 ± 242, 743 ± 146, 853 ± 418, and 679 ± 250 and total urine volumes (ml) over the 8-h exposure were 416 ± 419, 93 ± 126, 450 ± 452, and 131 ± 180 for SL-EUH, SL-HYPO, ALT-EUH, and ALT-HYPO, respectively, with HYPO values lower ($P = 0.06$) than EUH for urine volume.

Blood variables. Plasma fluid regulatory hormone responses are shown in Fig. 6. Results primarily indicate that there was a significant hydration effect, i.e., values were higher in HYPO vs. EUH trials for PRA, ALD, and AVP, with no differences between SL and ALT, whereas values in the HYPO trials were lower than EUH for ANP. Analysis of PRA also showed a significant altitude-by-hydration interaction, primarily caused by a higher 8-h value in SL-HYPO compared with the other trials. S100β was not different between hydration levels or altitude exposures (Fig. 7). Values for S100β before exposure ranged from 0.10 to 0.16 μg/l and after 8 h of exposure ranged from 0.18 to 0.22 μg/l.

DISCUSSION

This study was the first to experimentally evaluate the effect of hypohydration (hyperosmotic-hypovolemic) on aerobic exercise performance and the prevalence and severity of AMS symptoms during high-altitude exposure. Hyperosmotic hypovolemia was used as the model of hypohydration, caused by exercise-induced sweating, to mimic the type of hypohydration commonly observed in recreational and military activities. A moderate hypohydration level was employed, and a warm ambient temperature was selected to simulate a typical summer day at moderate altitude in equatorial regions. At sea level, moderate hypohydration will degrade aerobic performance in temperate, warm, and hot ambient conditions (25). The performance trial was selected to accentuate cardiovascular limitations (25), and a longer exercise bout would likely result in larger performance decrements by accentuating glycogen depletion and thermal strain (13). The principal findings from this study were *1)* the combination of hypohydration and altitude exposure to 3,048 m reduces aerobic exercise performance in an additive manner over hypohydration and altitude exposure alone; and *2)* hypohydration did not appear to increase the prevalence and severity of symptoms associated with AMS.

Hypohydration combined with 3,048-m altitude exposure caused a 34% decline in aerobic exercise performance while exercising in warm air. The combination of the two stressors was additive; independently hypohydration caused a 19% degradation and 3,048-m altitude exposure caused an 11% degradation in performance. Figure 2 clearly demonstrates that performance, relative to the SL-EUH trial, was significantly impaired in the other three trials. Further evidence for a practical importance for these findings is supported by the data showing that the 95% confidence limits for the SL-HYPO and ALT-HYPO trials lie outside the zone of indifference (defined as the coefficient of variation of the cycle ergometer time-trial test) and that the ALT-EUH trial confidence limits fall almost entirely outside the CV, synonymous with a significant differ-
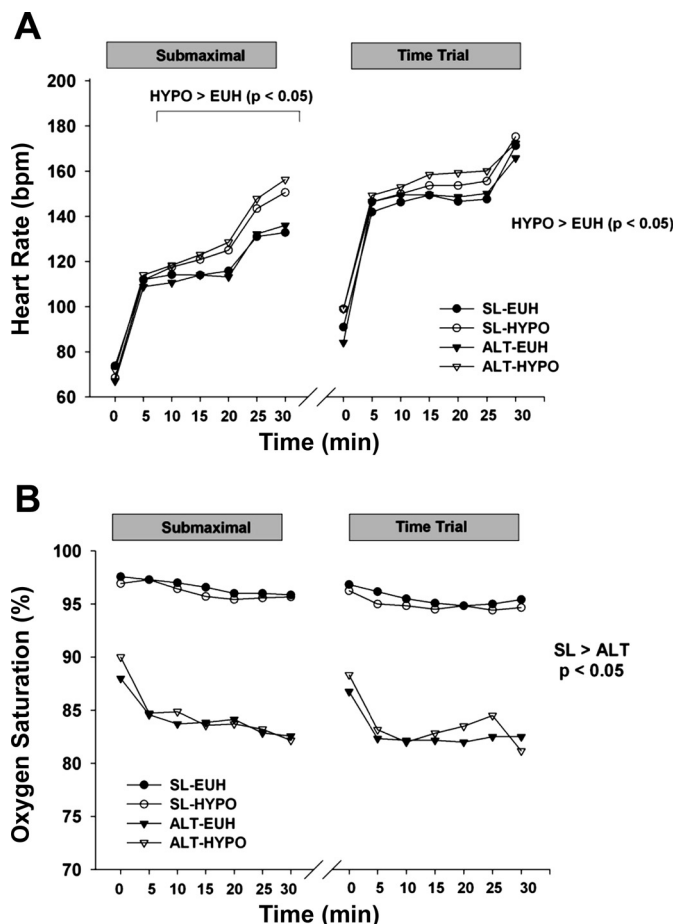

Fig. 5. Heart rate (*A*) and O$_2$ saturation (*B*) values vs. time during submaximal exercise and during the time-trial performance. Heart rates were higher during HYPO trials vs. EUH (main effect). O$_2$ saturation values were lower during ALT vs. SL trials. Range of SDs for HR: submaximal, 3.0–11.7 beats/min; time trial, 7.8–24.4 beats/min. Range of SDs for O$_2$ saturation: submaximal, 0.7–2.7%; time trial, 1.0–5.0%.

Table 3. *Thermal variables during submaximal exercise*

	SL		ALT	
	EUH	HYPO	EUH	HYPO
T_{core}, °C				
0 min	36.58 (0.13)	36.60 (0.47)	36.51 (0.24)	36.57 (0.19)
15 min	37.1 (0.38)	37.30 (0.24)	37.02 (0.21)	37.09 (0.21)
30 min	37.39 (0.41)	37.86 (0.38)	37.28 (0.21)	37.59 (0.30)
T_{skin},°C*				
0 min	32.08 (0.57)	32.56 (0.65)	32.51 (0.47)	32.21 (0.65)
15 min	31.68 (0.48)	32.12 (0.56)	31.47 (0.45)	31.73 (0.57)
30 min	32.07 (0.84)	32.31 (0.85)	31.62 (0.77)	31.67 (0.24)
SR, mg·min^{-1}·cm^{-2}				
0 min	0.09 (0.04)	0.09 (0.03)	0.09 (0.05)	0.11 (0.03)
15 min	0.22 (0.11)	0.19 (0.06)	0.17 (0.07)	0.19 (0.12)
30 min	0.26 (0.05)	0.27 (0.04)	0.23 (0.09)	0.23 (0.15)
CVC, %maximal				
5 min	13.7 (11.4)	17.1 (11.7)	15.2 (4.5)	12.8 (3.8)
15 min	30.5 (32.2)	30.4 (28.5)	31.1 (13.3)	24.1 (14.9)
25 min	30.5 (24.0)	41.2 (29.5)	41.5 (22.6)	31.3 (16.5)

Values are means (SD). T_{core}, core temperature; T_{skin}, mean skin temperature; SR, sweating rate; CVC, cutaneous vascular conductance. *Significant time-by-hydration interaction ($P < 0.02$) where HYPO > EUH throughout submaximal exercise.

ence, although not necessarily important. Furthermore, these data demonstrate that performance degraded to a similar extent at moderate HYPO as that observed at 3,048 m (10,000 ft). Many individuals quickly understand that altitude exposure degrades aerobic performance but fail to appreciate the same magnitude of performance degradation when hypohydrated.

Several mechanisms can be postulated for the large degradation in aerobic exercise performance during ALT-HYPO. ALT, independently, reduces submaximal exercise performance at 3,048 m, compared with SL, by 15–20% during events ranging from 20–30 min (18, 26). SaO_2 is reduced at 3,048 m (to 83% during the time-trial performance) compared
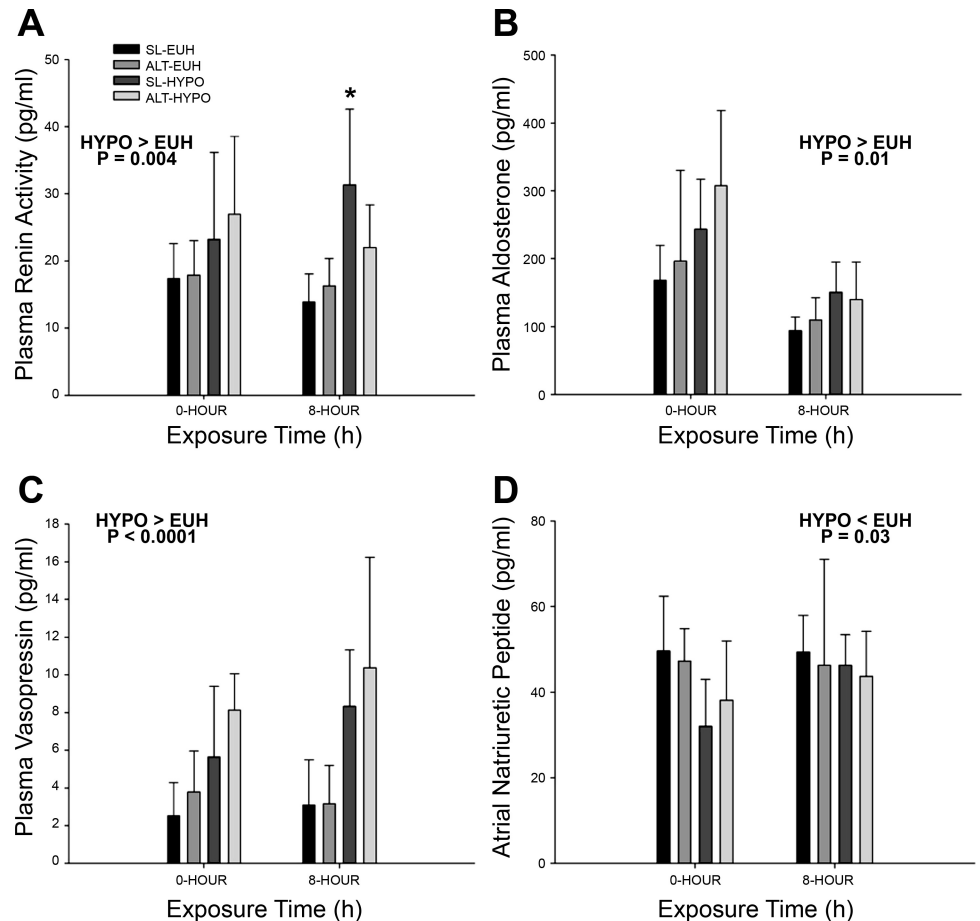

Fig. 6. Plasma renin activity (*A*), aldosterone (*B*), vasopressin (*C*), and atrial natriuretic peptide (*D*) before (0 h) and after (8 h) exposure for all trials. Values were higher (see individual graphs for *P* values) during HYPO vs. EUH trials for plasma renin activity, plasma aldosterone, and plasma vasopressin. For atrial natriuretic peptide, values were lower in HYPO vs. EUH. *8-h PRA value is greater ($P < 0.03$) than other 3 trials.
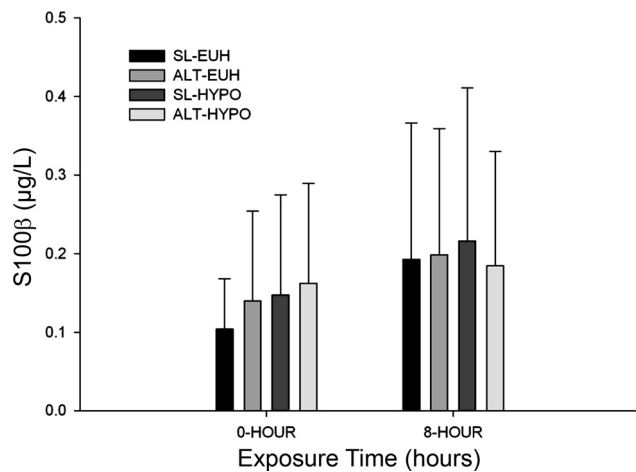
Fig. 7. Plasma S100beta before (0 h) and after (8 h) exposure for all trials. There were no differences among experimental trials.

with sea level (95%), lowering the muscle capillary-to-mitochondrial oxygen gradient and contributing to a decline in the rate of oxygen delivery from the blood to the muscle (17). Furthermore, since subjects began the time trial at a linear factor set at 50% of the sea-level $\dot{V}_{O_{2peak}}$, maintaining this power output at ALT to match the power output at SL would require subjects to work at a higher relative exercise intensity. Rather, subjects chose to reduce their power output at ALT, relative to the SL trials, by ~20 W.

Hypohydration degrades aerobic exercise performance at sea level in temperate/warm-hot conditions (11, 25). Mechanisms for this include increased cardiovascular and thermoregulatory strain, hypovolemia, and increased perceptual strain (13). Subjects' HRs were ~15–20 beats/min higher during submaximal exercise and 5–10 beats/min higher during the time trial when hypohydrated. Likewise, subjects' core temperatures were ~0.4°C higher during HYPO vs. EUH and mean skin temperatures were higher in HYPO. These changes, combined with the plasma volume reduction, likely reduce cardiac filling and cardiac output. Gonzalez-Alonso et al. (20, 21) have shown that increased HR, hyperthermia, and lower plasma volume reduce cardiac output in HYPO compared with EUH. Furthermore, it has been demonstrated that $\dot{V}_{O_{2max}}$ is reduced by hypovolemia (27, 36). We observed a 9.5% decline in PV before exposure in HYPO compared with EUH. In temperate environments, a 4–8% decline in maximal aerobic power occurs after HYPO by 3% (23a). Thus during submaximal exercise in a HYPO state, subjects work at a higher relative exercise intensity and either become fatigued sooner or have to reduce their power output, as observed here. Subjects also perceived that they were working harder during HYPO, which may have also contributed to a reduction in performance. These data suggest that the large degradation in aerobic performance when exposed to 3,048-m altitude and hypohydrated is due to the additive effects of a reduction in arterial oxygen content, stroke volume, cardiac output, and $\dot{V}_{O_{2peak}}$ and an increase in cardiovascular, thermal, and perceptual strain.

This study was the first to prospectively examine the prevalence and severity of symptoms of AMS at 3,048 m over an 8-h time period with hyperosmotic hypohydration. When hypohydrated, AMS prevalence increased 2.5 times and severity scores by 2.5 times, but these increases were not statistically significant. There was greater variability in the altitude sickness scores when the subjects were hypohydrated and the number of subjects was not sufficient to observe significance.

Our findings are supported by Aoki and Robinson (1). In the only study to dehydrate subjects before hypobaric hypoxia before a prolonged altitude exposure (≥ 8 h), Aoki and Robinson (1) found no deleterious effect of 2.5% dehydration on AMS, using furosemide to induce an isosmotic hypovolemia (compared with hyperosmotic hypovolemia in present study). In contrast, other studies (7, 14, 30, 31) found that dehydration may worsen the symptoms of AMS. Two of these reports were epidemiological/field studies (7, 14) and the others examined AMS symptoms after only 100 min of hypoxic exposure. Although our findings were similar to Aoki and Robinson (1), we did observe a tendency for higher symptom severity scores with HYPO and the volunteers reported feeling sicker, which has important applications to field environments, although we acknowledge that we cannot distinguish between symptoms associated with AMS and HYPO (see Fig. 4B, where 2 subjects were "diagnosed" with AMS during SL-HYPO using the LLS; one subject had AMS in all trials). Further studies with larger numbers of subjects appear warranted to determine if hyperosmotic hypohydration indeed does increase AMS. Perhaps the nonsignificant elevation we observed in AMS severity scores was due to the type of dehydration induced. Using a diuretic, as Aoki and Robinson (1) used, will cause intravascular and interstitial volumes to decrease proportionately, causing no change in plasma solute concentrations, whereas dehydration induced by exercise in the heat increases plasma solutes and decreases intracellular fluid (osmotic gradient pulling fluids from tissues) to a greater extent than isosmotic hypovolemia. Specific studies comparing these two types of dehydration are warranted.

In summary, 4% hypohydration and 3,048-m exposure combine to additively reduce aerobic exercise performance. The performance degradation when these two stressors were combined (−33%) was equivalent to the sum of the performance degradations induced independently by hypohydration and altitude exposure. Hypohydration tended to increase the prevalence and severity of AMS symptoms, but not statistically. These findings strongly suggest that dehydration be avoided before ascending to high altitude such that further physical performance decrements are minimized.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

1. **Aoki VS, Robinson SM.** Body hydration and the incidence and severity of acute mountain sickness. *J Appl Physiol* 31: 363–367, 1971.
2. **Armstrong LE, Costill DL, Fink WJ.** Influence of diuretic-induced dehydration on competitive running performance. *Med Sci Sports Exerc* 17: 456–461, 1985.
3. **Bailey DM, Evans KA, McEneny J, Young IS, Fall L, Gutowski M, Kewley E, McCord JM, Moller K, Ainslie PN.** Altered free radical metabolism in acute mountain sickness: implications for dynamic cerebral autoregulation and blood-brain barrier function. *J Physiol* 587: 73–85, 2009.
4. **Bailey DM, Taudorf S, Berg RMG, Lundby C, McEneny J, Young IS, Evans KA, James PE, Shore A, Hullin DA, McCord JM, Pedersen BK, Moller K.** Increased cerebral output of free radicals during hypoxia: implications for acute mountain sickness? *Am J Physiol Regul Integr Comp Physiol* 297: R1283–R1292, 2009.
5. **Bartsch P, Maggiorini M, Schobersberger W, Shaw S, Rascher W, Girard J, Weidmann P, Oelz O.** Enhanced exercise-induced rise of aldosterone and vasopressin preceding mountain sickness. *J Appl Physiol* 71: 136–143, 1991.
6. **Bartsch P, Shaw S, Franciolli M, Gnadinger MP, Weidmann P.** Atrial natriuretic peptide in acute mountain sickness. *J Appl Physiol* 65: 1929–1937, 1988.
7. **Basnyat B, Lemaster J, Litch JA.** Everest or bust: a cross sectional, epidemiological study of acute mountain sickness at 4243 meters in the Himilayas. *Aviat Space Environ Med* 70: 867–873, 1999.
8. **Beidleman BA, Muza SM, Fulco CS, Rock PB, Cymerman A.** Validation of a shortened electronic version of the environmental symptoms questionnaire. *High Alt Med Biol* 8: 192–199, 2007.
9. **Borg GAV.** Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med* 2: 92–98, 1970.
10. **Cheuvront SN, Bearden SE, Kenefick RW, Ely BR, DeGroot DW, Sawka MN, Montain SJ.** A simple and valid method to determine thermoregulatory sweating threshold and sensitivity. *J Appl Physiol* 107: 69–75, 2009.
11. **Cheuvront SN, Carter R, Castellani JW, Sawka MN.** Hypohydration impairs endurance exercise performance in temperate but not cold air. *J Appl Physiol* 99: 1972–1976, 2005.
12. **Cheuvront SN, Carter R, Sawka MN.** Fluid balance and endurance exercise performance. *Curr Sports Med Rep* 2: 202–208, 2003.
13. **Cheuvront SN, Kenefick RW, Montain SJ, Sawka MN.** Mechanisms of aerobic performance impairment with heat stress and dehydration. *J Appl Physiol* (August 5, 2010). doi:10.1152/japplphysiol.00367.2010.
14. **Cumbo TA, Basnyat B, Graham J, Lescano AG, Gambert S.** Acute mountain sickness, dehydration, and bicarbonate clearance: preliminary field data from the Nepal Himalaya. *Aviat Space Environ Med* 73: 898–901, 2002.
15. **Dill DB, Costill DL.** Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 37: 247–248, 1974.
16. **Ebbut AF, Frith L.** Practical issues in equivalence trials. *Stat Med* 17: 1691–1701, 1998.
17. **Fulco CS, Lewis SF, Frykman PN, Boushel R, Smith S, Harman E, Cymerman A, Pandolf KB.** Muscle fatigue and exhaustion during dynamic leg exercise in normoxia and hypobaric hypoxia. *J Appl Physiol* 81: 1891–1900, 1996.
18. **Fulco CS, Rock PB, Cymerman A.** Maximal and submaximal exercise performance at altitude. *Aviat Space Environ Med* 69: 793–801, 1998.
19. **Gagge AP, Stolwijk JAJ, Hardy JD.** Comfort and thermal sensations and associated physiological responses at various ambient temperatures. *Environ Res* 1: 1–20, 1967.
20. **Gonzalez-Alonso J, Mora-Rodriguez R, Below PR, Coyle EF.** Dehydration markedly impairs cardiovascular function in hyperthermic endurance athletes during exercise. *J Appl Physiol* 82: 1229–1236, 1997.
21. **Gonzalez-Alonso J, Mora-Rodriguez R, Coyle EF.** Stroke volume during exercise: interaction of environment and hydration. *Am J Physiol Heart Circ Physiol* 278: H321–H330, 2000.
22. **Graichen H, Rascati R, Gonzalez RR.** Automatic dew-point temperature sensor. *J Appl Physiol* 52: 1658–1660, 1982.
23. **Hoyt RW, Honig A.** Body fluid and energy metabolism at high altitude. In: *Handbook* of Physiology. Environmental Physiology. Bethesda, MD: Am. Physiol. Soc., 1996, sect. 4, vol. II, chapt. 55, p. 1277–1289.
23a.**Institute of Medicine**. Water. In: *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and sulfate*. Washington, DC: Institute of Medicine 2005, p. 73–185.
24. **Kanner AA, Marchi N, Fazio V, Mayberg MR, Koltz MT, Siomin V, Stevens GH, Masaryk T, Aumayr B, Ayumar B, Vogelbaum MA, Barnett GH, Janigro D.** Serum S100beta: a noninvasive marker of blood-brain barrier function and brain lesions. *Cancer* 97: 2806–2813, 2003.
25. **Kenefick RW, Cheuvront SN, Palombo LJ, Ely BR, Sawka MN.** Skin temperature modifies the impact of hypohydration on aerobic performance. *J Appl Physiol* 109: 79–86, 2010.
26. **Mazzeo RS, Fulco CS.** Physiological systems and their response to conditions of hypoxia. In: *ACSM's Advanced Exercise Physiology*, edited by Tipton CM. Philadelphia: Lippincott Williams and Wilkins, 2005, p. 564–580.
27. **Nybo L, Jensen T, Nielsen B, Gonzalez-Alonso J.** Effects of marked hyperthermia with and without dehydration on V̇o₂ kinetics during intense exercise. *J Appl Physiol* 90: 1057–1064, 2001.
28. **Raff H.** Endocrine adaptations to hypoxia. In: *Handbook of Physiology*. Environmental Physiology. Bethesda, MD: Am. Physiol Soc., 1996, sect. 4, vol. II, chapt. 54, p. 1259–1275.
29. **Ramanathan ML.** A new weighting system for mean surface temperature of the human body. *J Appl Physiol* 19: 531–533, 1964.
30. **Richardson A, Watt P, Maxwell N.** Hydration and the physiological responses to acute normobaric hypoxia. *Wild Environ Med* 20: 212–220, 2009.
31. **Richardson A, Watt P, Maxwell N.** The effect of hypohydration severity on the physiological, psychological and renal hormonal responses to hypoxic exercise. *Eur J Appl Physiol* 106: 123–130, 2009.
32. **Roach R, Stepanek J, Hackett PH.** Acute mountain sickness and high-altitude cerebral edema. In: *Medical A*spects of Harsh Environments, edited by Pandolf KB, Burr RE. Falls Church, VA: Office of the Surgeon General, U.S. Army, 2002, p. 765–793.
33. **Roach RC, Bartsch P, Oelz O, Hackett PH; Lake Louise AMS. Scoring Committee.** *The Lake Louise Acute Mountain Sickness Scoring System*, edited by Sutton JR, Houston CS, Coates G. Burlington, VT: Queen City, 1993, p. 272–274.
34. **Roach RC, Maes D, Sandoval D, Robergs RA, Icenogle M, Hinghofer-Szalkay H, Lium D, Loeppky JA.** Exercise exacerbates acute mountain sickness at simulated high altitude. *J Appl Physiol* 88: 581–585, 2000.
35. **Sampson JB, Kobrick JL, Johnson RF.** Measurement of subjective reactions to extreme environments: the environmental symptoms questionnaire. *Mil Psych* 6: 215–233, 1994.
36. **Sawka MN.** Physiological consequences of hydration: exercise performance and thermoregulation. *Med Sci Sports Exerc* 24: 657–670, 1992.
38. **Sawka MN, Young AJ, Pandolf KB, Dennis RC, Valeri CR.** Erythrocyte, plasma, and blood volume of healthy young men. *Med Sci Sports Exerc* 24: 447–453, 1992.